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Transplantation in Rats

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The work reported in this paper is a realization of an idea first obtained in a college course of zoology. The unusual regenerative power that the invertebrates and some animals as high as amphibians have, led me to believe that similar powers might be present in a lesser degree in warm-blooded animals as well. For several years the idea was borne in mind that it should be possible to transplant parts from one individual to another in higher animals as well as in lower ones if the proper conditions were supplied. The solution came when in medical school we were made acquainted with what has been done in transferring large pieces of tissue from one part of an animal to another, such as transferring a portion of a dog's stomach to the surface of its body. It seemed that if one could transfer a large piece of tissue from one location to an entirely different one on the same animal by maintaining a nourishing pedicle, it should be just as possible to reflect the pedicled piece of tissue to an entirely different animal. Instead of transferring a flap of tissue to a new location on the same animal why not attempt putting the flap onto a new animal maintained in close proximity? With these ideas in mind, portions of legs and tails were found to be transferable from one animal to another in the same fashion that parts of stomachs were moved about upon the body of a dog.

I wish to express profound gratitude to Dr. Essenberg whose scientific attitude and approach has enkindled in me the spark of a similar outlook upon life. To him I also owe the opportunity for being able to work out an idea that lay dormant in my mind for several years. I wish to express my appreciation to Dr. Strong for his able guidance throughout the entire year, and specifically for his assistance in the preparation and acceptance by the publishers of my first scientific paper. Many thanks are also due to Dr. Job and Dr. Hughes for criticism and corrections of this paper as well as for Dr. Job's acting as the jovial host at our seminar luncheons. To Mr. Warren I owe my knowledge of tissue preparation and staining techniques.

The transplantation of legs in amphibians was referred to in a previous paper (Schwind, 1936). Weiss has transplanted legs to various locations on salamanders and obtained quite measurable amounts of motion in the new limbs. Similar successful transplantations in animals as high as mammals have not hitherto been reported.

Transplantation of a part as large as a leg from one rat to another is made possible by giving special attention to the maintenance of an adequate nutritional and oxygen supply to all the cells in the part being transplanted. The blood supply can not be completely interrupted even for a short period of time. In the transfer of a leg, this continuous blood supply was maintained by a parabiotic union of the animal donating its leg, to the animal receiving the leg. For convenience in description the process will be described as it was employed in the replacement of a completely amputated limb. Fig. 1 is a diagrammatic sketch of three steps in this operation of replacing an amputated hind leg. In the first step is shown the animal (b) whose right hind leg has been removed and discarded, and the animal (a) which is going to give up its left hind leg to (b). The leg which is to be transplanted is partially amputated through the middle of the thigh, as indicated by the dotted lines. A triangular piece of flesh is removed from the lateral aspect of the thigh to allow the stump of the amputated leg of the recipient to fit deeply into the soft tissues of the new leg. At the same time as this partial amputation from the donor is done, the femur of this leg is cut completely through just above the knee joint, and also the sciatic nerve is cut so as to leave an end long enough to facilitate its attachment to the sciatic nerve of the recipient.

The second step in the operation as seen in Fig. 1 shows how the leg is attached to the recipient by its lateral half, and to its original owner by the medial half. The distal cut end of the femur in this leg is now attached to the stump of the originally amputated femur of the recipient's leg. Connection of the cut ends of these bones was effected by boring a hole through each of the two ends to be united and then putting a suture through these two holes in the manner seen in Fig. 2a. A small splint of fresh rat bone was made to fit snugly into the approximated open ends of the bones, and then the suture was drawn tight as in Fig. 2b. In this same step the proximal cut end of the sciatic nerve of the recipient was united to the distal cut end of the sciatic nerve of the leg being transplanted. Union of these nerve ends was made in the manner seen in Fig. 3. The approximated cut ends were split lengthwise in all planes so that when they were placed beside each other, many of the free fibers of the proximal and would interdigitate with the free fibers of the distal end, and possibly grow new nerve fibers out into the distal end. A very fine suture was tied about the two nerve ends as they were placed side by side. Sutures sufficiently fine for this type of work had to be devised, as the smallest size on the market was many times too large. It was found that quite satisfactory suture material could be made from rat tail tendons, an idea devised and perfected by a medical student, E. L. Schrey. The tendons are taken from the tail and dried for at least a day. Then they can be split to any desirable size. Thus, from one rat's tail several score of very find tendons of quite suitable tensile strength can be obtained.

The muscle mass on the extensor side of the stump of the recipient's amputated leg was joined to the extensor muscles of the new leg. In a similar manner the flexor muscles of the new leg were tied into the flexor muscles of the recipient's stump. Skin flaps were drawn over the leg, covering all open surfaces.

When the animals were properly joined, as in the second step of the operation, several layers of adhesive tape were put about the bellies of the two animals, to act as an immobilizing device. The legs were all left free, so the animals could walk about, - however, there was little motion between the two animals. A union of nine days was found to be quite sufficient to allow the leg to grown on to the recipient, and success has even been obtained with only six days union. The animals to be united were chosen at about twelve days of age. It is necessary to remove them from the mother as she bites off the bandages. Animals younger than twelve days survive only with difficulty if they are removed from the mother and fed artificially with an eyedropper.

When the leg appears to be adequately healed to its new host, it is cut off from its original owner. This process of removing it from the original owner involves merely amputating the remaining medial half as the lateral half had been cut in the first part of the operation. Cutting this medial half in one operation was not found to be as practicable as cutting in several successive steps at separate intervals. Although there are many small blood vessels connecting the new leg to the host, they are incapable of carrying sufficient blood to maintain the life of the leg if all the vascular connections to the original owner are suddenly severed. Completion of the amputation from the donor must be done gradually so as to create a physiological need in the leg for more blood. This makes the new vessels that have grown out from the recipient dilate enough to answer the need. So during the last three days of the parabiotic union a little more of the remaining connection is cut each day, until only a small flap of skin and other soft tissue remains on the last day, and this is cut through as the last step in severing the donor from its leg. This leaves the recipient with a new hind leg attached to the stump of its previously amputated left hind leg. Thus it now has two left hind legs.

The same principles are employed in transplanting a fifth leg to the back of an animal, only in this case the new leg is partially united to a denuded surface on the back of the animal and allowed to develop a connective tissue and vascular attachment at this point. In the sketch in Fig. 4 is seen the manner in which the lateral branch of the right sciatic nerve was cut and reverted around the back to the location of the new leg. In animals killed after several months, the nerve can be seen and dissected out. Fig. 7 is a photograph of one of these nerves as it is seen in its new location turning dorsally to join with the extra leg. In the lower right corner of the picture are seen the animals own hind legs. The right one of these contributed the nerve seen running dorsal toward the fifth leg which appears in the upper left corner of the picture. Microscopic sections show the nerve to be apparently normal both axis cylinders and myelin sheaths being quite clear.

Fig. 5 is a photograph of the posterior part of the body of a rat possessing an extra hind leg on its back. This is the same animal that was reported in a previous article as being able to flex the ankle of the new leg with sufficient force to lift a ten gram weight a distance of one centimeter. The animal is still living at the date of writing, thirteen months after transplantation, but the flexion movements have become very slight. Daily massage and exercise have been discontinued for several months.

Fig. 6 is a photograph of the anterior part of a rat with an extra front leg. The new leg is the one being held by the forceps seen in the lower right corner of the picture. This leg was innervated by a branch of the left brachial plexus. A slight flexion of the toes of this new leg could be elicited by making the animal flex the toes of its own left front leg, from which a nerve had been taken for the new leg. When the animal was made to flex the toes of its left front leg, the toes of the new leg flexed in unison.

Fig. 8 is a picture of another animal with an extra leg upon its back similar to the one previously described. However, no apparent motion was ever observed in this leg. Two other attempts at transplanting legs to backs of animals resulted in only slight muscular control of the new leg. Replacement of amputated legs by new ones was attempted, and at the date of writing one of these animals has been walking upon its new legs for three months. However there is no apparent ability to use the toes in this case. Although the leg is used in walking, it has not been determined how much of the motion is due to muscle pulls exerted by the animals own muscles and how much is due to the action of the transplanted muscle.

The technique used in these leg transplants was worked out first by transplanting parts of tails from one animal to another. It was found to be possible to transplant a piece of tail to a second animals back and to various other locations such as the one seen in Fig. 9. No nerves were directed into these tails at the time of transplantation, but in every case a very definite sensibility to pain developed. Sensory nerve fibers find their way out into these new parts, as is very well evidenced by the fact that pinching or bending the new tail causes the animal to jump and emit a sound of pain. Transplantation of parts of tails has been previously reported by Bert, this work also having been done on rats.

The method of eliciting a response in an extra leg transplanted to the back is worthy of some comment. Pinching the leg which contributed the lateral branch of its sciatic nerve causes motion in the new leg which received the nerve. The most successful stimulation was found to be pinching the gastrocnemius tendon. This caused a sudden flexion at the ankle of the leg grafted to the back.

Several transplanted legs were allowed to grow for a period of time and then were removed for sectioning and staining. The first of these legs was removed two and a half months after transplantation and a detailed study of the tissue was made after it was sectioned and stained with hematoxylin and eosin. Fig. 10 shows a low power microphotograph of a section of skin and subcutaneous tissue. There is apparently no difference between these transplanted tissues and normal tissue. Microscopically all the layers of the epidermis can be made out. Scattered about in the subcutaneous tissue are the numerous hair follicles, and an examination of each follicle shows it to have all the root sheaths in the same manner as any normal hair. The hair on the transplanted legs grows at the same rate as the normal hair on other parts of the body. Both the transplanted hair and skin seem to be well oiled and a microscopic study of the hair roots shows definite sebaceous glands opening into the hair follicles. In the upper part of the center of the first microphotograph (Fig.10) can be seen a hair follicle that was cut longitudinally. There is a small sebaceous gland opening into the base of the follicle. The second microphotograph (Fig. 11) shows the oil gland under high power. In the upper center part of the picture can be seen the root of the hair shaft and arranged about it are the large, clear-staining, oil gland cells. Tiny droplets of oil are quite evident in the cytoplasm of the gland cells and are seen as a fine reticulation or stippling in the photograph.

Another skin structure that apparently did not suffer from the transplantation is the toe-nail. Toe-nails of legs which were put upon the backs of animals grew to be very long. Being placed upon the back, the toes were not walked upon and consequently were not kept shortened by the wearing-off process of this walking. Some of these transplanted toe-nails have grown so long that they formed a complete semi-circle and the distal end touched the bottom of the toe.

A histological study of the muscle tissue in the legs reveals the fact that the muscle fibers have lost neither the staining characteristics of their nuclei nor the cross bands or striations. Fig. 12 is a high power microphotograph of some of these skeletal muscle fibers two and one-half months after transplantation. The dark and light discs or cross striations are evident and the light line of Hensen can be seen within the dark disc, as well as the dark line of Krause within the light disc. In cross-sectional cuts of the individual muscle fibers can be seen the bundles of myofibrillae or Cohnheim's areas. The individual muscle fibers are somewhat smaller in diameter than normal ones, and cell nuclei are more abundant in the grafted muscle.

A decalcified specimen of one transplanted leg was prepared for the purpose of studying the bone and marrow in microscopic preparations. Fig. 13 is a high power microphotograph of a section of this bone and marrow. Bone cells can be seen enclosed in the lacunae of the bone in the right third of the picture. Since the bone as a whole has grown in size along with the general body growth, the osteoblasts apparently are functional. Within the shaft of the bone are seen various types of cells which tend to indicate that active hemopoiesis is occurring in the transplanted marrow tissue. Different stages of myelocytes or young forms of heterophils, eosinophils and basophils can be seen with their kidney-shaped nuclei. Under the microscope erythroblasts and many erythrocytes are evident. In the upper left hand corner of the microphotograph are two megakaryocytes or giant cells, and two more are apparent in the lower left part of the picture near the center.

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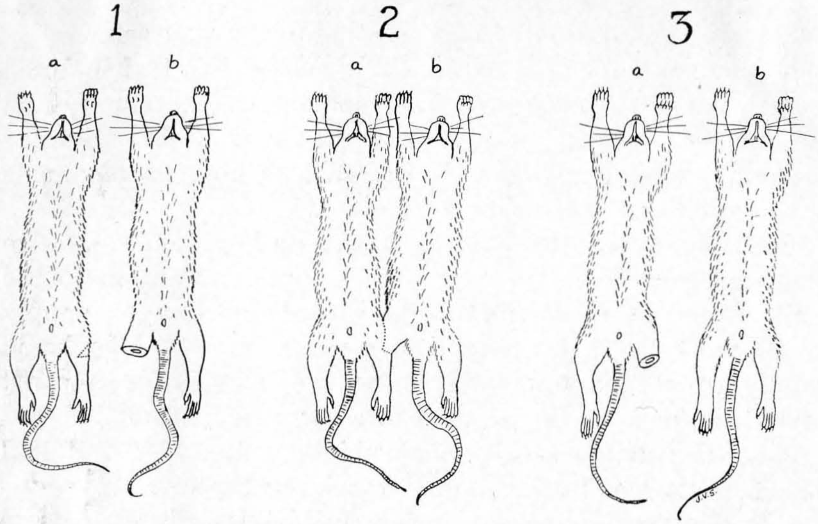


Fig. 1. Transplantation for leg replacement.

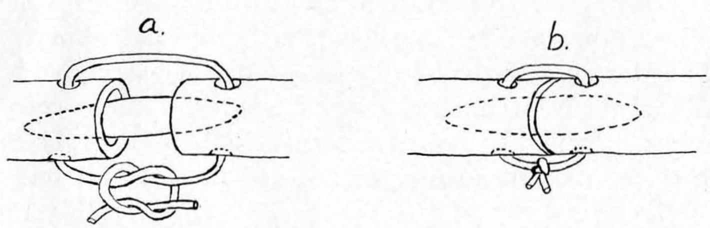


Fig. 2. Bone union detail.

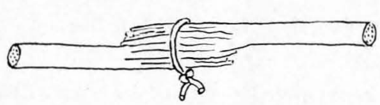


Fig. 3. Nerve union detail.

Fig. 4. Fifth leg innervation.

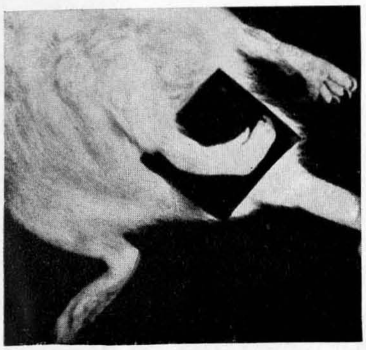


Fig. 5 Fifth leg on back.

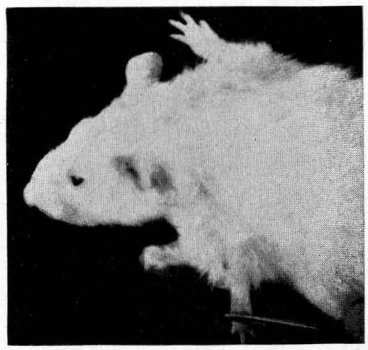


Fig. 6 Fifth leg on shoulder.

Fig. 7 Nerve to fifth leg on back.

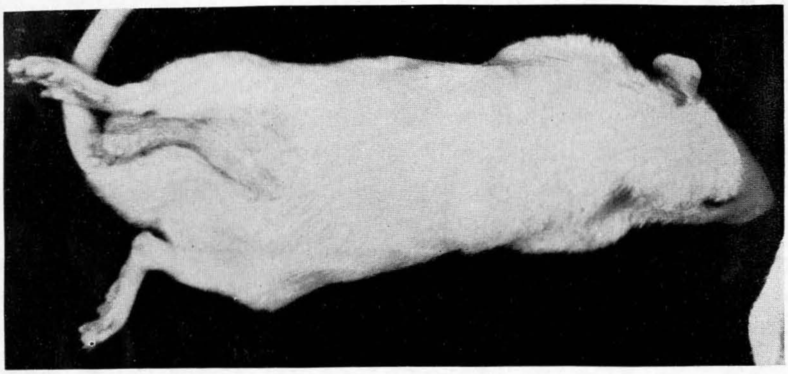


Fig. 8 Fifth leg on back.

Fig. 9 Extra tail on neck.

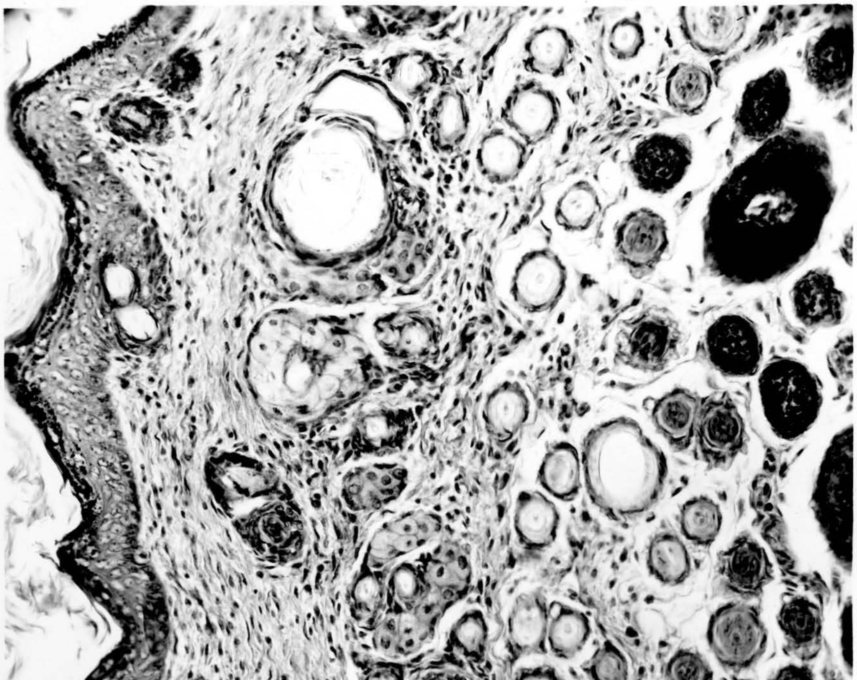
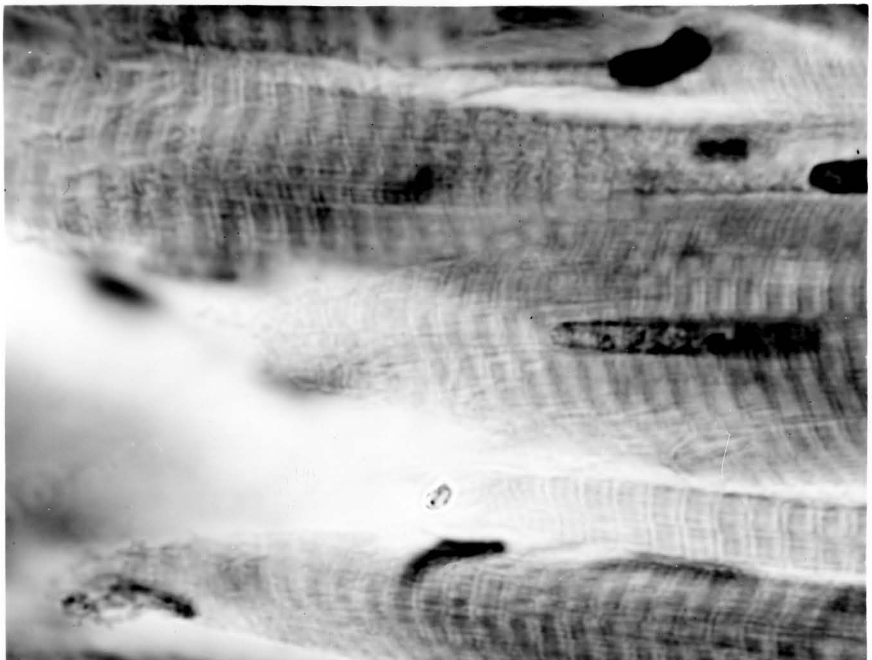


Fig. 10. Skin, subcutaneous tissues and hair follicles.

Fig. 11. Single hair follicle with oil gland at base.



. Fig. 12. Fibers of skeletal muscle.

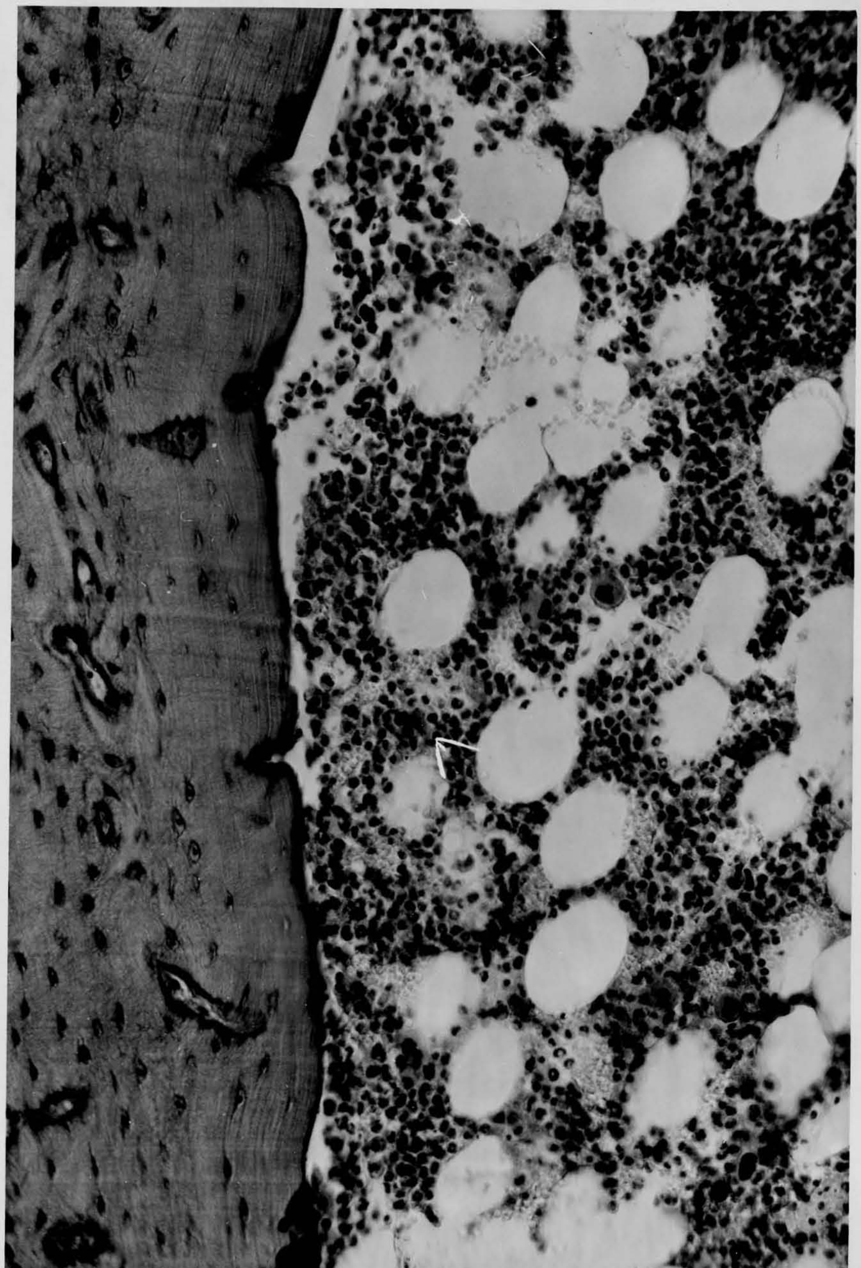


Fig. 13. Bone and bone marrow.